The Effect of Radioactive Phosphorus on the Development of *Aedes aegypti* Larvae[1]

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INTRODUCTION

The technique of eradication of insects by the release of sterile males is well known now after its successful application to screw-worm flies in Curacao Island. The possibility of applying this technique to other insects is being considered by many workers. So far it has not been successful in the complete eradication of mosquitoes but McCray and Jensen (1961) found that a dose of 10,500–17,500 r from a cobalt-60 source was sufficient to sterilize the males.

Phosphorus^{32} was used for the studies of dispersal and mating behavior in *Anopheles* mosquitoes by Quraishi, Lamsachi and Ergul (1962) and Quraishi and Arthur (1963) in this laboratory. No change was observed in the behavior of adults or in egg laying after larvae were reared with 10 to 20 μc per litre of P^{32} in the medium. This activity gave satisfactory counting rates. Hassett and Jenkins (1957) studied uptake of P^{32} by larvae from comparatively high concentrations of P^{32} ranging from 50 to 5000 μc per litre. Egg laying and mating were normal up to 1000 μc per litre. Bruce-Chwatt and Hayward (1956) on the other hand found that a smaller number of adults emerged at concentrations above 100 μc per litre.

The present investigation was designed to examine the rate of uptake of P^{32} from solution by larvae of *Aedes aegypti* and its effect on their development. Mosquito larvae concentrate P^{32} to a high degree (Hassett and Jenkins); thus the dose actually delivered is considerably greater than would be supposed if only the concentration in the solution were considered. The possibility of giving a sterilizing dose by this means was examined. The method offers the advantages that the dose is more uniform and since accumulation of phosphorus is greater in and near the gonads (Hassett and Jenkins), local irradiation may be more intensive. The application of P^{32} in the breeding solution would avoid the relatively heavy capital cost and elaborate shielding necessary for irradiation with cobalt-60.

MATERIALS AND METHODS

A pure strain of *Aedes aegypti* was reared from 4 pairs of mosquitoes from a $1^1/_2$-year inbred colony. The colony was maintained in a specially

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constructed large box with the temperture thermostatically controlled at 27°C. It was found that this greatly assisted the hatching of larvae. In the experiments reported in detail, freshly hatched larvae up to 24 hours old were used. Normally 50 larvae were used for each treatment. Carrier free radioactive phosphorus was added to solutions. The volume varied from 100–250 ml. Details of the experiments are given in Table I. Two hundred mg of pablum food were added to each dish. The dishes were kept in an incubator at 27°C.

TABLE I. *Concentration of P^{32} and number of larvae in the rearing trays*

Exp. No.	Concentrtion of P^{32} (μc/litre)	Volume of solution (ml)	Total activity (μc)	Number of larvae
3	15.6	250	3.90	40
	4.8		1.20	
	1.32		0.33	
4	38.7	250	9.67	50
	8.04		2.01	
5	38.7	250	9.68	50
	7.56		1.89	
6	57.2	100	5.72	50
	52.6		5.26	
7	94.7	150	14.2	50
	89.3		13.4	

Each day a 1 ml sample of the solution was taken and dried on a planchet for counting. The samples were collected up to the day of pupation. Samples of 3 larvae were also collected each day in experiments 4 and 5, up to the time of pupation. The larvae were rapidly filtered and washed with carrier solution and distilled water before mounting on a planchet for counting.

In each experiment, pupae were removed from the medium as they formed and were kept individually in large tubes until emergence. Adult mosquitoes were killed with chloroform and prepared for counting.

P^{32} was assayed by means of an end-window counter, calibrated with a standard source. All results are presented corrected for decay.

RESULTS

Experiments 1 and 2 are not reported in detail, since they were preliminary in nature and were designed only to examine techniques.

The decline in the concentration of P^{32} in the solution during the period of larval development is plotted in Fig. 1 for experiments 4, 6 and 7. With the exception of experiment 6, the activity was removed by the larvae in an approximately exponential manner. The removal of P^{32} from the medium and the measured uptake in the larvae was in reasonable agreement in

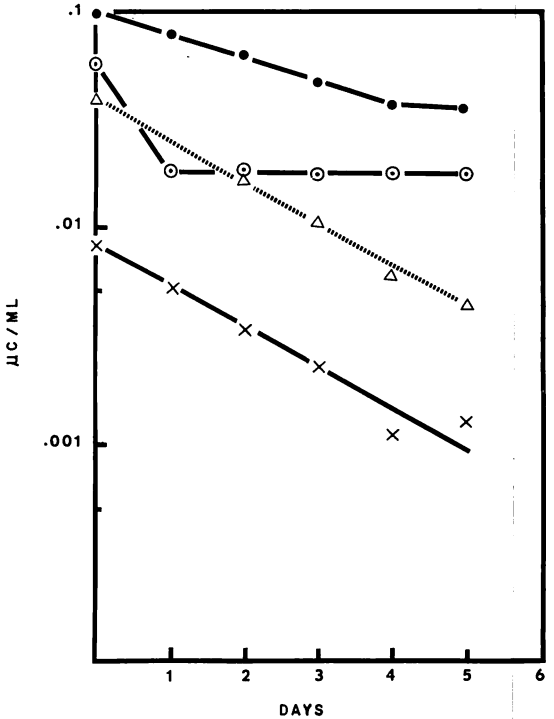

Fig. 1. *Removal of P^{32} from solution by developing larvae of* Aedes aegypti. *Key to experiments:* △ △ 4, 9.7 μc in 25 ml; × × 4, 2.0 μc in 250 ml; ⊙ ⊙ 6, 5.5 μc in 100 ml; ● ● 7, 13.8 μc in 150 ml.

the two experiments in which both measurements were made. The results of experiment 4 are given in Fig. 2; similar results were obtained in experiment 5.

The amount of P^{32} found in adult mosquitoes is shown in Table II. Approximate concentration-factors based on the initial concentration have been calculated on the assumption that the amount of P^{32} in pupae and adults was the same and that the average volume of the pupae was 4 mm^3. The concentration-factors ranged from 400–1800 in females and from 400–800 in males, the females in each case being twice as radioactive as the males. Within experiments, similar concentration factors were achieved from different initial concentrations. Between experiments the concentration-factors differed appreciably. The lower factors in experiments 6 and 7 are attributed to the lower volume of solution which was used; and the smaller total amount of P^{32}, rather than concentration, limited the amount which could be taken up by the larvae.

The effect of the radioactive phosphorus on the development of the larvae was also observed. A delay in the growth of 3rd-instar larvae was observed in concentrations above 15 μc per litre. Concentrations of

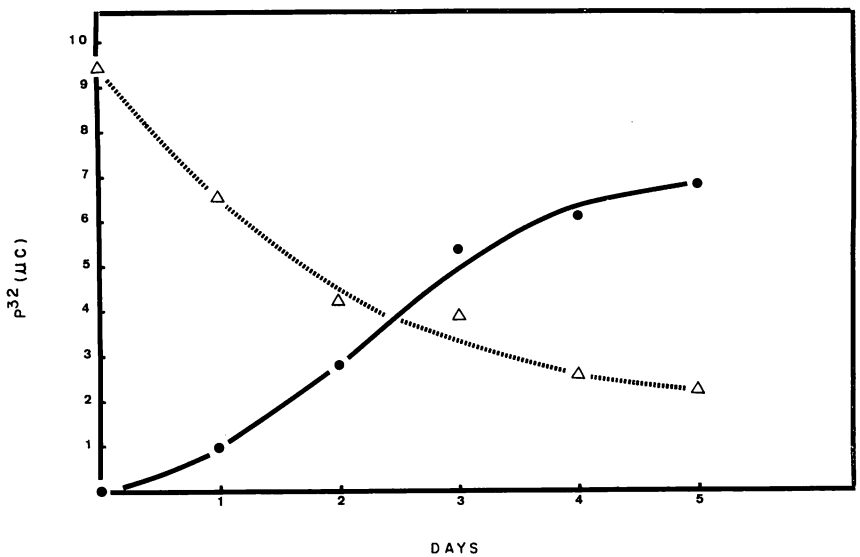

Fig. 2. *A comparison of uptake by larvae and loss from the solution of* P^{32}. *Key to experiments:* ● ● *Larval uptake*; △ △ *Solution loss.*

TABLE II. *Activity in adult mosquitoes*

Exp. No.	Initial activity in solution (mμc/ml)	Activity in females (mμc)	Number measured	Concentration factor*	Activity in males (mμc)	Number measured	Concentration factor*
3	15.6	69.1	3	1110	46.1	7	740
	4.8	24.5	6	1250	12.5	3	650
	1.32	4.27	5	815	1.98	8	375
4	38.7	246.0	2	1588	114.0	8	730
	8.04	47.8	13	1488	26.5	6	825
5	38.7	223.0	12	1443	106.0	4	685
	7.56	54.7	5	1828	21.5	16	710
6	52.6	96.4	9	458	43.5	8	207
	57.2	90.1	3	395	52.4	8	229
7	94.7	241.0	17	635	91.0	4	240
	89.2	211.0	15	590	80.2	10	225

*Calculated on the assumption that the amount of P^{32} in adults and pupae is the same and that the average volume of the pupae is 4 cubic mm.

38.7 μc per litre and upwards delayed the achievement of the 4th-instar stage by about one day. This led to a subsequent delay of 24 hours in pupation and emergence of the adult mosquitoes as compared to the control.

Mating and egg laying between active males and normal females was examined in experiments 6 and 7. Egg laying was delayed by one day after mating with radioactive males in both the experiments. The number of eggs laid as a result of this mating was reduced to one-fifth of the control

in experiment 6 and one-half of the control in experiment 7 (Table III). The eggs in both these experiments produced larvae. In experiment 6 the adult males were very inactive and failed to fly. A proportion failed to emerge or survive.

TABLE III. *Number of eggs layed after mating with normal and radioactive males*

Exp. No.	Days after mating	Number of eggs layed*	
		A†	B‡
6	3	105	550
	4	245	1095
7	3	0	358
	4	776	1250

* Eggs from these matings produced larvae
†A Radioactive males with normal females
‡B Normal males and females

DISCUSSION

The experiments described above showed that larvae of *Aedes aegypti* were able to concentrate P^{32} to a very high degree. Apparently, when carrier free solutions are used as in the present experiments, the concentration-factor is not dependent on the initial level in the solution and the exhaustion of the solution caused the number of larvae per ml of solution to be the limiting factor in concentration. Hassett and Jenkins (1951) reported that larvae of *Aedes aegypti* concentrated P^{32} at least 75 times above the level of the medium. The values reported here are considerably greater.

At levels of P^{32} of 50–100 μc/litre the production of eggs by normal females mated with radioactive males was reduced compared with normal matings. This suggests that sterilization might be effected by the addition of P^{32} to the rearing medium. On the other hand, the levels of P^{32} effective in partially sterilizing the mosquitoes caused a delay in larval development, pupation and adult emergence. Further experimentation is necessary if the balance between sterilization and reduction in viability is to be established.

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